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Helicobacter pylori-Induced Gastritis in the Domestic Cat

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Helicobacter pylori **has been cultured from the inflamed gastric mucosae of naturally infected cats; the lesions in *H. pylori*-infected cat stomachs mimic many of the features seen in *H. pylori*-infected human stomachs. To determine whether *H. pylori*-negative specific-pathogen-free cats with normal gastric mucosae were susceptible to colonization by this bacterium and whether gastritis developed after infections, four *H. pylori*-negative cats treated with cimetidine were orally dosed three times with 3 ml (1.5×10^8 CFU/ml) of *H. pylori* every 4 days. All four cats became persistently colonized as determined by gastric cultures and PCRs from serial gastric biopsy samples and necropsy samples at 7 months postinfection. *H. pylori* was not isolated from the two control cats, nor were their gastric tissues positive by PCR; one of the two cats had a few focal lymphocytic aggregates in the body submucosa, whereas the second cat had a normal gastric mucosa. All four *H. pylori*-infected cats had multifocal gastritis consisting of lymphoid aggregates plus multiple large lymphoid nodules, which were most noticeable in the antral mucosa. In addition, one *H. pylori*-infected cat had a moderate diffuse infiltration of polymorphonuclear leukocytes in the subglandular region of the antrum. *H. pylori*-like organisms were focally distributed in glandular crypts of the antrum. Two of the *H. pylori*-infected cats had significant (eightfold) increases over baseline in levels of immunoglobulin G *H. pylori* serum antibody. The *H. pylori* isolates from the four experimentally infected cats had restriction fragment length polymorphism patterns specific for the *flaA* gene that were identical to those of the inoculating strain. *H. pylori* readily colonizes the cat stomach and produces persistent gastritis.**

Since the discovery in 1983 by Warren and Marshall of *Helicobacter pylori* as the cause of active chronic gastritis, this new gastric pathogen has been firmly established as the major etiological agent of peptic-ulcer disease, and it is now strongly linked to development of gastric adenocarcinoma and gastric lymphoma (9, 13, 18, 19, 24, 26, 37, 40, 42, 43, 52). Although gastric spiral organisms have been observed in dogs and cats for over 90 years, their presence in these species has been largely ignored and they were sometimes cited as gastric commensals (3, 23, 50). However, because of the tremendous interest in *H. pylori* as a gastric pathogen, renewed interest has been focused on gastric organisms in animals, the role of these organisms in producing gastric pathology, and their potential as zoonotic gastric pathogens. Animals also have been the subjects of experiments designed to study the pathogenesis and epidemiology of *H. pylori*. Unfortunately, attempts to infect a variety of animal species with *H. pylori* were unsuccessful, with the exception of studies with gnotobiotic and (to a lesser extent) specific-pathogen-free pigs, gnotobiotic dogs, Old World macaque species, and most recently, athymic or germfree mice (2, 27, 28, 30, 44). Furthermore, early attempts to isolate *H. pylori* from domestic animals were unsuccessful; the only studies in which *H. pylori* was isolated from the stomachs of naturally infected animals were restricted to macaques, particularly rhesus monkeys, *Macaca mulatta* (2, 4, 6, 39).

We recently cultured *H. pylori* from a group of commercially reared cats (20, 21). The organism was isolated from inflamed gastric tissues of these cats and was observed histologically in 100% of examined cats from the same source. The purpose of this study, therefore, was to establish whether the *H. pylori* feline strain could experimentally infect naive cats, establish a persistent infection, and elicit significant gastritis and a serum antibody response.

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MATERIALS AND METHODS

Animals. Six specific-pathogen-free, male cats, aged ~8 months, were obtained from a commercial vendor; these six animals and other cats from this vendor's barrier-maintained facility previously tested for gastric *Helicobacter*-like organisms (GHLOs) were found to be free of these bacteria (20, 21).

The cats were housed in animal facilities accredited by the American Association for Accreditation of Laboratory Animal Care. The cats were individually maintained in stainless steel cages during a 4-week quarantine. After being dosed, the four cats receiving *H. pylori* were housed for 1 month in individual cages and then were group housed in a room (10 by 10 ft [ca. 300 by 300 cm]) to enhance socialization of the animals. To minimize any potential of microbial cross-contamination, the two control cats were placed in a separate building, where they lived in a stainless steel cage and were provided free access to an enclosure (6 by 6 ft [ca. 200 by 200 cm]). All cats were examined daily and given food (Iams Cat Diet; Iams Co., Dayton, Ohio) and water ad libitum.

Bacterial inoculation. *H. pylori* 94-2728, isolated from a cat with gastritis, was used for oral inoculations (20). The organism was grown in brucella broth for 72 h at 37°C under microaerophilic conditions.

Enzyme-linked immunosorbent assay (ELISA). For antigen preparation, four strains of *H. pylori* isolated from cats were grown for 72 h microaerophilically on Trypticase soy agar–5% blood agar plates at 37°C. Bacteria were harvested and suspended in sterilized phosphate-buffered saline (PBS), and the suspension was centrifuged at 10,000 rpm (ca. $9,000 \times g$) for 10 min. The pellet was resuspended in PBS and sonicated until bacterial cell membranes were disrupted. The preparation was centrifuged at 3,000 rpm (ca. $800 \times g$) for 10 min, the resulting supernatant was collected, and its protein concentration was determined by the Lowry method (36). The antigen preparation was then aliquoted and stored at −70°C.

The ELISA was based on our previously published methods (12, 13). Each well of a flat-bottom Immulon II plate (Dynatech, Chantilly, Va.) was coated with 100 ng of antigen preparation in a volume of 100 μl per well in 0.1 M carbonate buffer, pH 9.6. The antigen-coated plates were stored at 4°C overnight. The wells were then washed four times with PBS–0.05% Tween 20, the plates were dried, and the wells were filled with PBS–1% bovine serum albumin (BSA) and incubated at 37°C for 1 h. The plates were again washed four times with PBS and were dried, and 100-μl volumes of serum at dilutions ranging from 1:64 to 1:2,048 were added to the wells. The serum was diluted in PBS–1% BSA. The plates

TABLE 1. Experimental *H. pylori* infection in cats

Cat no.	Result at time postdosing[a]																			
	2 wk				4 wk				8 wk				12 wk				24 wk			
	Culture		PCR		Culture		PCR		Culture		PCR		Culture		PCR		Culture		PCR	
	A[b]	B[c]	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
9	−	−	+	−	+	+	+	−	−	−	+	+	+	+	−	−	+	+	−	+
10	−	−	−	−	+	+	−	−	+	+	+	−	+	+	−	−	+	+	−	−
11	−	+	+	−	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
12	−	−	−	−	+	+	+	−	c[d]	c	+	+	−	−	−	−	−	+	+	+

[a] Control cats 7 and 8 had *H. pylori*-negative gastric biopsy samples at each time point.
[b] A, antral biopsy sample.
[c] B, body biopsy sample.
[d] c, contaminated sample.

were then incubated at 37°C for 1 h and were rerinsed and dried; 100 μl of anti-cat immunoglobulin G (IgG)-peroxidase (Jackson Immuno Research Laboratory, West Grove, Pa.) at a 1:25,000 dilution with PBS was added, and the plates were incubated at 37°C for 1 h. After the plates had been rerinsed and dried, 100 μl of ABTs peroxidase substrate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) was added to each well and incubated for 15 min at room temperature. The optical density at 450 nm was then read on a model MR 5000 Dynatech Microplate reader. Positive control serum (2642) from a cat naturally infected with *H. pylori* and negative control serum from four cats with *H. pylori*-negative culture results were included in every assay. Serum with a titer of <1:64 was considered negative.

Experimental design. Food was withheld overnight from four specific-pathogen-free cats; the following morning they were weighed and cimetidine (10 mg/kg) was administered intramuscularly to induce temporary hypochlorhydria. After 1 h, the cats were anesthetized and 3 ml of broth containing 1.5×10^8 CFU of *H. pylori* per ml (as measured by the plate dilution technique) was inoculated into the stomach of each cat via an orogastric tube. All four cats received the same inoculum on days 5 and 8 of the experiment. Cimetidine was administered subcutaneously or intramuscularly twice a day throughout the inoculation period until 2 days following the third inoculation (10 days total).

At 2 weeks prior to infection and at scheduled intervals (2, 4, 8, 12, and 24 weeks) after dosing, gastric biopsy samples were obtained from all six cats (four infected and two control) for culture, PCR, and histology (Table 1). While the cats were under anesthesia, mucosal samples from the bodies and antra (three from each region) were obtained via gastroscopy with a 4.9-mm-diameter Pentax pediatric bronchoscope. This instrument has monoplanar control of the tip and a single open lumen to accommodate flexible pinch-biopsy forceps or be used for insufflation, irrigation, and suction as required. One sample each from the antrum and the body was processed individually for culture, PCR, and histology.

Venipuncture was performed, blood was collected, and sera were processed for all six cats prior to initiation of the study and at 2, 4, 8, 12, 24, and 30 weeks postinoculation (p.i.). Sera were stored at −70°C prior to the ELISA.

All six cats were anesthetized at 7 months p.i., blood was collected, and the animals were euthanized with an intracardiac injection of a euthanasia solution containing pentobarbitol (Somlethol). Necropsy samples from three antral, two body, and one cardia site from each cat stomach were cultured for *H. pylori*; PCR analysis was performed with samples from one site each from the antra, bodies, and fundi of two infected and two control cats, whereas for the other two *H. pylori*-infected cats, two sites from each of these three areas of the stomach were analyzed for the presence of *H. pylori* by PCR (Fig. 1).

Microbiology. Gastric biopsy and necropsy samples were processed within ~1 h. Each biopsy sample was homogenized with a sterile tissue grinder and inoculated onto Trypticase soy agar–5% sheep blood agar plates (BBL, Cockeysville, Md.) and brucella blood agar plates containing antibiotics (trimethoprim, vancomycin, and polymyxin B; Remel, Lenexa, Kans.). The plates were incubated for 3 to 7 days at 37°C under microaerophilic conditions in vented jars containing N_2, H_2, and CO_2 (90:5:5). Bacteria were identified as *H. pylori* by Gram stain; morphology under phase microscopy; strong oxidase, catalase, and urease positivities; resistance to nalidixic acid; and susceptibility to cephalothin (20).

DNA extraction of tissue. Gastric biopsy and necropsy sections were minced and suspended in STET buffer (8.0% sucrose, 50 mM EDTA, 0.1% Triton X-100, and 50 mM Tris-HCl [pH 8.0]). Lysozyme (from hen egg white; Boehringer-Mannheim, Indianapolis, Ind.) was added to a final concentration of 3 mg/ml, the solution was incubated for 12 min at 37°C, and sodium dodecyl sulfate was added to a final concentration of 1.0%. Samples were incubated for 1 h in 50 mg of RNase (Boehringer-Mannheim) per ml and then subjected to overnight digestion in pronase (0.8 mg/ml) and proteinase K (0.5 mg/ml). The DNA was then extracted in phenol-chloroform (1:1) and precipitated overnight at −20°C in the presence of 0.3 M sodium acetate and 2.1 volumes of absolute ethanol. The samples were centrifuged at 15,000 × *g* for 30 to 60 min, air dried, and resuspended in sterile distilled water.

PCR amplification for *H. pylori*. The selection of primers for PCR amplification was based on previously published data detailing the specificity and sensitivity of these primers (25). Primer U3 contained the sequence (5′ to 3′) CAG CAG CCG CGG TAA TAC, covering bases 518 to 535 in *Escherichia coli* 16S rRNA; primer Hp1 contained the sequence (5′ to 3′) CTG GAG AGA CTA AGC CCT CC, covering bases 834 to 853 in *E. coli* 16S rRNA.

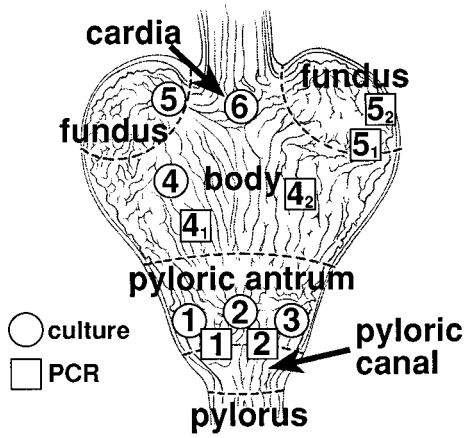

FIG. 1. Schematic depicting gastric mucosal sites from which samples were taken at necropsy for *H. pylori* culture and PCR. The numbers correspond to site numbers in Table 2.

TABLE 2. *H. pylori* culture and PCR results for cat gastric samples taken at necropsy

Cat no.	Result(s) at site[a]									
	Antrum			Body			Fundus			Cardia, 6
	1	2	3	4	4.1	4.2	5	5.1	5.2	
Infected										
9	+, (+)	+, (+)	−	+	(+)	(+)	−	(+)	(+)	+
10	−, (+)	−, (−)	−	+	(+)	(+)	+	(+)	(+)	+
11	−, (+)	+	+	−	(+)		+	(+)		+
12	+, (+)	+	+	−	(+)		−	(−)		+
Control										
7	−, (−)	−	−	−	(−)		−	(−)		−
8	−, (−)	−	−	−	(−)		−	(−)		−

[a] + and −, positive and negative results by culture, respectively. (+) and (−), positive and negative results by PCR, respectively. PCR samples for sites 4.1, 4.2, 5.1, and 5.2 were taken from sites different from culture sites; otherwise PCR samples were taken from the same sites as gastric biopsy samples used for culture. The locations of the sites are shown in Fig. 1.

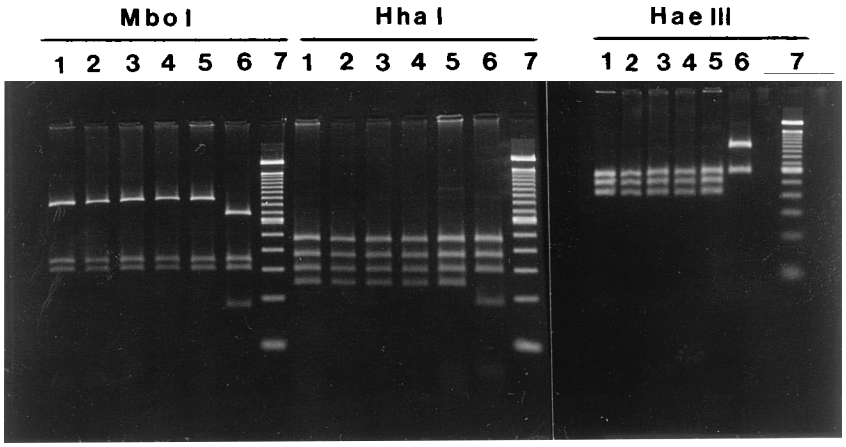

FIG. 2. RFLP analysis of the PCR-amplified 1.5-kb *flaA* segment of *H. pylori* with the indicated restriction enzymes. Lanes 1, PCR product from the infecting cat *H. pylori* strain; lanes 2 to 5, PCR products from strains recovered from the experimentally infected cats; lanes 6, PCR products from an *H. pylori* strain different from that in lane 1; lanes 7, 100-bp molecular size markers (Gibco BRL, Gaithersburg, Md.).

Fifty nanograms to 5 μg of each DNA preparation was added to a reaction mixture (1× *Taq* polymerase buffer, 2.25 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl [pH 8.4], 0.1% [wt/vol] gelatin, 0.5 μM each primer, 200 μM each deoxynucleotide, and 2.0 mg of BSA per ml) with a final volume of 100 μl. The samples were heated to 94°C for 5 min to denature the DNA and were then centrifuged and cooled to 61°C. *Taq* polymerase (Boehringer-Mannheim) (2.5 U) and 1.0 U of polymerase enhancer (Perfect Match; Stratagene, La Jolla, Calif.) were then added to each reaction tube and then overlaid with 100 μl of mineral oil. The following conditions were used for amplification: denaturation of the DNA template at 94°C for 1 min, annealing at 61°C for 1.75 min, and elongation at 72°C for 1.75 min. A total of 33 cycles were performed and were followed by a final elongation step at 72°C for 8.0 min. PCR products were subjected to electrophoresis on a 6.0% Visigel separation matrix (Stratagene) followed by ethidium bromide staining and viewing under UV illumination.

PCR amplification and restriction fragment length polymorphism (RFLP) analysis. Primer sequences chosen for amplification were specific for the *H. pylori flaA* gene (1, 47). The two oligonucleotides, 5′ATGGCTTTTCAGGTC AATAC and 5′GCTTAAGATATTTTGTTGAACG, produced an amplified product of 1.5 kb. Phenol-chloroform-extracted DNA (1 to 20 ng) was added to a reaction mixture with a final volume of 100 μl as indicated elsewhere (47). The samples were heated to 95°C for 5 min, briefly centrifuged, and then cooled to 60°C, at which time 2.5 U of *Taq* polymerase and 1 U of polymerase enhancer were added and then overlaid with 100 μl of mineral oil. This was followed by a reannealing step of 2.0 min at 60°C and an elongation step of 2.25 min at 72°C. Thirty-four additional cycles comprising 1.0 min at 94°C, 2.0 min at 60°C, and 2.25 min at 72°C were carried out; these were followed by a final elongation step of 7 min at 72°C. Following PCR amplification, 15 μl of reaction mixture was removed and digested with 10 U of restriction enzyme for 4 h as outlined by the manufacturer (Boehringer-Mannheim) and electrophoresed for 1 h at 100 V through a 6.0% Visigel separation matrix.

Histopathology. A 1-cm-wide strip from the greater curvature of the stomach extending from the esophagus-cardia junction to the antrum-duodenum junction was collected from each of the two control and four experimental cats. Each specimen was carefully laid flat between layers of absorbent paper and immersed in neutral buffered 10% formalin. The strips were trimmed, processed by standard methods, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin stain or Warthin-Starry silver stain. Gastric biopsy samples taken during the course of the experiment were processed in the same manner.

RESULTS

Clinical signs. All six cats were without clinical signs during the quarantine and throughout the study.

Microbiology. All six cats were negative for *H. pylori* by both culture and PCR of gastric endoscopic biopsy samples collected prior to inoculation. Five sets of gastric biopsy samples taken from the antrum and body were collected sequentially and cultured for *H. pylori* throughout the course of the experiment (Table 1). Although each of the four experimentally infected cats did not have positive *H. pylori* culture results at each time interval, *H. pylori* was isolated from gastric biopsy samples from each of the four cats (either antral or body) at 4 weeks p.i. At 2 weeks p.i. only one cat was *H. pylori* positive, whereas at 8 and 12 weeks p.i. two of the cats were positive for *H. pylori* in a minimum of one gastric site and at 24 weeks p.i. three of the cats were *H. pylori* positive by culture. In the aggregate, 8 of 20 antral and 12 of 20 body gastric biopsy samples were positive for *H. pylori* by culture.

H. pylori was isolated from the gastric mucosae of all four infected cats at necropsy (7 months p.i.) but not from the two control cats. A minimum of three of the six biopsy sites for each infected cat yielded samples that were positive at necropsy (Table 2). For three of the cats, four biopsy sites yielded samples that were positive at necropsy. Interestingly, *H. pylori* was isolated from the cardia of all four infected cats. For three of the four cats, *H. pylori* was cultured from sites in both the antrum and the body.

PCR of cat gastric biopsy tissue. Control cats were uniformly negative by PCR for *H. pylori*. All four infected cats were intermittently positive by PCR at selected sites in gastric tissues from the antrum and body during the study. Collectively, 9 of 20 and 5 of 20 antral and body biopsy samples, respectively, were positive for *H. pylori* over the course of the experiment (Table 1).

At necropsy, 16 of 18 sites sampled from the four *H. pylori*-infected cats were positive for *H. pylori* by PCR (Table 2).

RFLP analysis of *H. pylori*. The identities of the strains of *H. pylori* isolated from the experimentally infected cats were compared by RFLP analysis of the 1.5-kb *flaA* gene segment with

TABLE 3. Histologic observations of gastritis in *H. pylori*-infected and control cats[a]

Cat no.	No. of lymphoid aggregates at site			No. of lymphoid nodules at site		
	Cardia	Body	Antrum	Cardia	Body	Antrum
Infected						
9	1	0	5	0	0	11
10	1	0	8	0	0	10
12	0	2	7	0	1	15
11	0	2	6	0	3	13
Control, 7	2	3	1	0	0	0

[a] Control cat 8 showed neither lymphoid aggregates nor nodules at any of the sites listed.

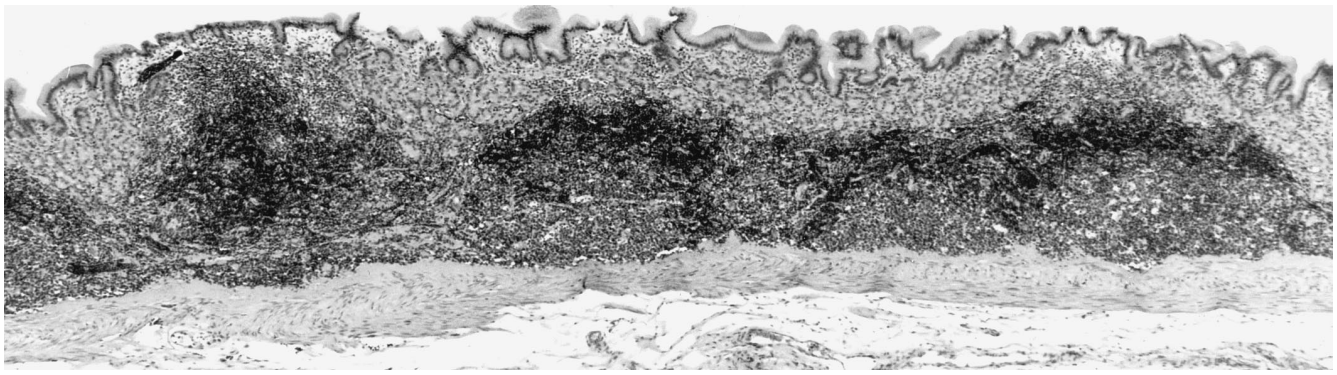

FIG. 3. Coalescing lymphoid follicles with germinal centers accompanied by severe glandular atrophy in the antral mucosa of cat 11, which was experimentally infected with *H. pylori*. Shown is a hematoxylin-and-eosin-stained tissue section. Magnification, ×36.

that of the infecting strain by using three enzymes, *Hae*III, *Mbo*I, and *Hha*I. With all three restriction enzymes, the RFLP pattern of the *flaA* gene PCR product of the infecting strain was virtually identical to those of strains isolated from the experimentally infected cats. Moreover, the patterns for all five *H. pylori* strains differed from that observed with an unrelated *H. pylori* human isolate (Fig. 2).

Histopathology. Biopsy samples from the stomachs of control animals were normal throughout the experiment. The gastric biopsy samples of the bodies and antra of the infected cats revealed only focal aggregates of mononuclear and polymorphonuclear inflammatory cells which were not consistently present throughout the study.

At necropsy, there were no significant histologic lesions observed in any portion of the gastric mucosa of one control cat. The second control cat had a mild focal mixed leukocytic infiltrate in the submucosa at the junction of the esophagus and cardia of the stomach, focal small aggregates of lymphocytes in the subglandular region of the distal portion of the body mucosa, and a similar small aggregate at the junction of the antral mucosa and the duodenum (Table 3).

In contrast to the findings for the control cats, each of the

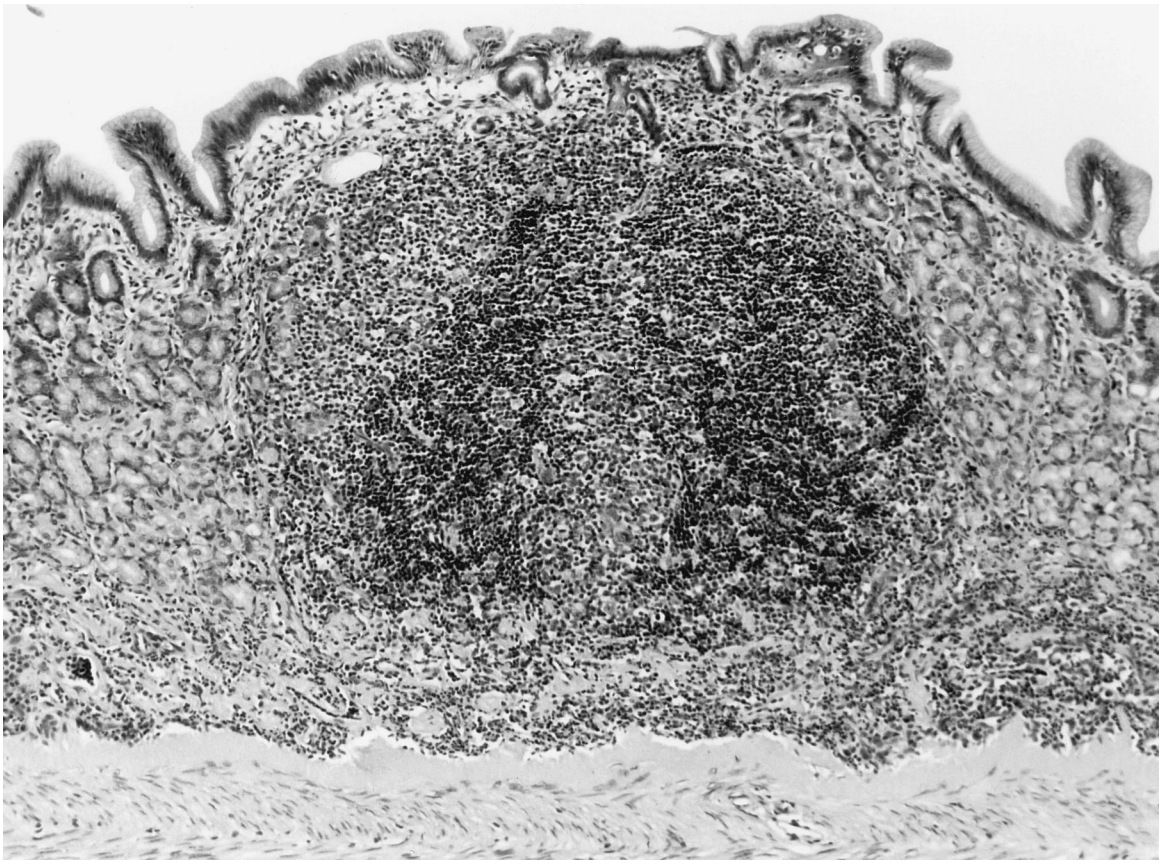

FIG. 4. Lymphoid nodule with a well-developed germinal center. The nodule was found in the antral mucosa of cat 12, which was infected with *H. pylori*. Shown is a hematoxylin-and-eosin-stained tissue section. Magnification, ×93.

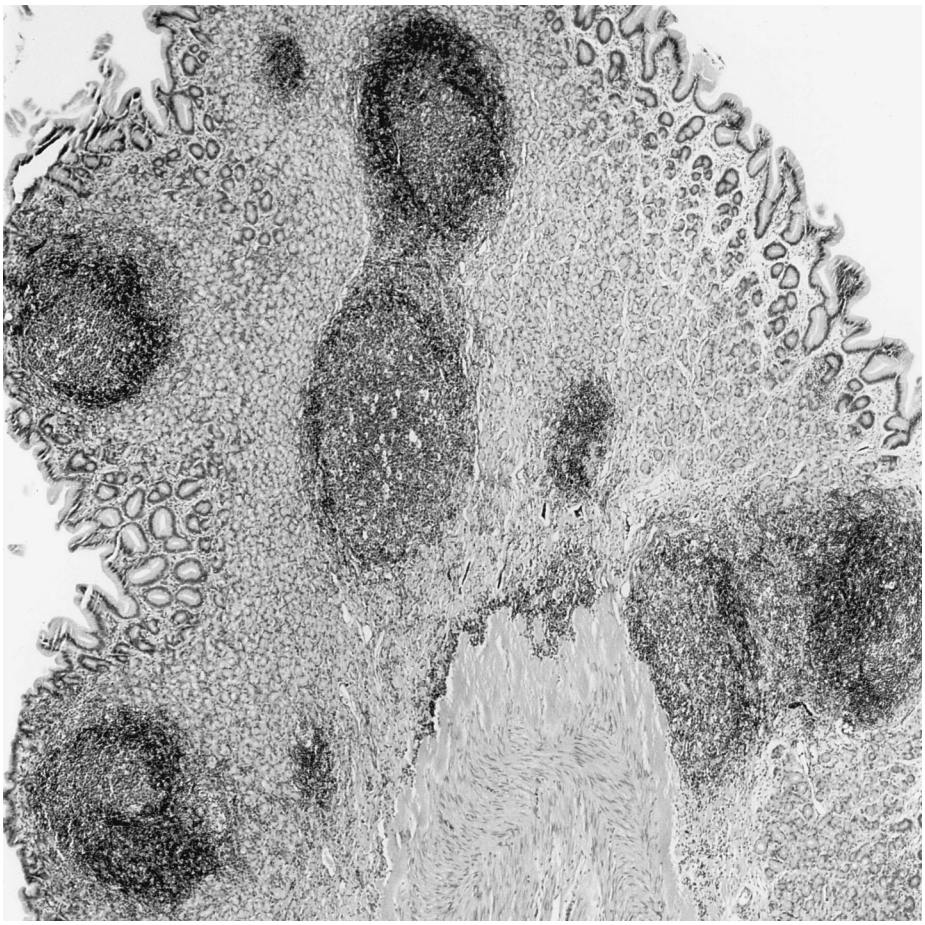

FIG. 5. Large lymphoid nodules observed in the antral mucosa of cat 11, which was infected with *H. pylori*. Shown is a hematoxylin-and-eosin-stained tissue section. Magnification, ×37.

experimentally infected cats had multiple small aggregates of lymphocytes plus multiple large lymphoid nodules with frequent germinal centers that extended through the full thickness of the mucosa and displaced glands (Fig. 3 and 4). The small lymphoid aggregates were located either in the subglandular portion of the mucosa or in the lamina propria at the level of the gastric pits. There were between six and nine of these small aggregates in each of the cats, and although a few were found in the cardia and body mucosa, the majority were concentrated in the antrum. The large lymphoid nodules were all found either in the distal portion of the body mucosa near the junction with the antral mucosa or within the antral mucosa (Fig. 5). For the infected cats, between 10 and 16 of these large lymphoid nodules were present in each gastric section examined (Table 3). In addition to these lesions, one infected cat had a moderate, diffuse infiltration of polymorphonuclear leukocytes with moderate numbers of eosinophils located in the subglandular region of the middle third of the antral mucosa. Small numbers of organisms with the morphology of *H. pylori* were detected by Warthin-Starry stain in the crypts of a few glands in the antrum (Fig. 6).

ELISA. Two experimentally infected cats showed twofold increases in *H. pylori* IgG titer by 2 weeks p.i. and greater than eightfold increases in titer by 20 weeks p.i. (Fig. 7). Two cats, however, had only slight increases in IgG titer, which were detected only after the two cats had been infected with *H. pylori* for 6 months. The two control cats had titers of ≤1:64 at each time point.

DISCUSSION

We have previously documented the presence of *H. pylori* in 100% of cats obtained from one of four commercial sources (20, 21). The organisms isolated were confirmed as *H. pylori* by morphologic and biochemical evaluation and by fatty-acid and 16S rRNA sequence analysis (20). The 16S rRNA sequence of cat isolate DO1 was 99.7% similar to that of the type strain of *H. pylori*. The cat sequence differed by 5 bases (of 1,475 bases), which is typical of the minor variations seen with other sequences of *H. pylori* available for analysis from GenBank (7, 20). Also, using primers specific to human *H. pylori* genes *ureA* and *ureB*, we were able to amplify similar-sized products from cat strains. RFLP analysis of these gene fragments confirmed the presence of regions conserved between cat and human strains (21). In further genotypic studies carried out with primers derived from other known genes homogeneous to *H. pylori* and which are of human origin, similar-sized products from all *H. pylori* isolates from the domestic cat were amplified. Equally important, phenotypic studies of *H. pylori* isolates from both cats and humans have confirmed the strains' similarity by determining antigenic relatedness (unpublished data).

For the naturally infected cats, histology and electron mi-

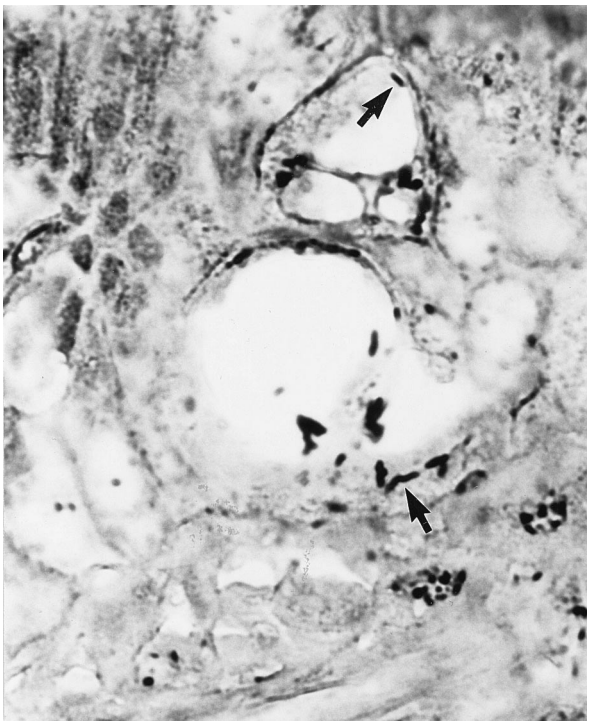

FIG. 6. *H. pylori* (indicated by arrows) in crypts of antral glands in cat 12, which was experimentally infected with *H. pylori*. Shown is a Warthin-Starry-stained tissue section. Magnification, ×930.

croscopy showed that numerous *H. pylori* cells in gastric tissue adhered to the gastric epithelium or were present in the mucous layers of the glandular or surface epithelium (20, 21). These cats had mild to severe diffuse lymphoplasmacytic infiltrates in the subglandular and mucosal tissues of the antrum. Because the domestic cat could be a significant reservoir host of *H. pylori* for zoonotic transmission to humans and because appropriate animal models are needed to study the pathogenesis and epidemiology of disease caused by *H. pylori*, we wanted to fulfill Koch's postulates by showing that *H. pylori* is

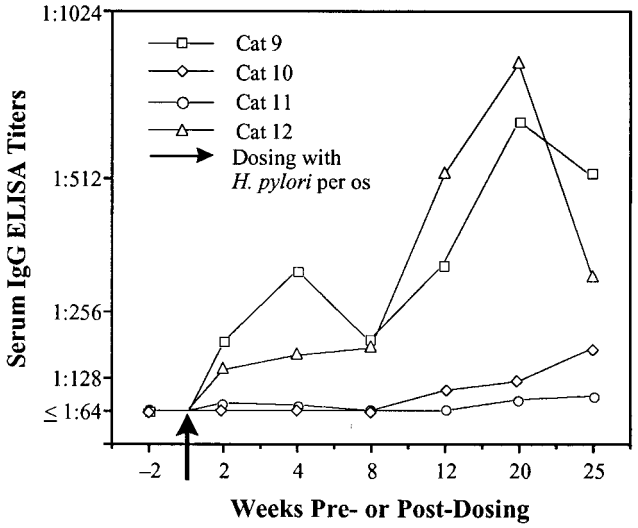

FIG. 7. *H. pylori* IgG serum antibody titers as measured by ELISA in four cats experimentally infected with *H. pylori*.

a gastric pathogen in cats. Fortunately, we had previously identified cats that were ideally suited for this experimental transmission study because they were free of *H. pylori* and other GHLOs. Like the control cats in this study, these cats had either no gastritis or minimal focal gastritis (20, 21).

Oral dosing with *H. pylori* in four cats receiving cimetidine during dosing produced persistent infection in all four cats. However, during the course of the experiment, not all biopsy samples from individual *H. pylori*-infected cats were *H. pylori* positive by culture and/or PCR. These findings are compatible with the focal nature of the organisms in the cats as determined histologically. A lack of 100% positivity by culture and/or PCR of biopsy samples from infected cats is consistent with results from human studies which have used a combination of histology, serology, culture, and (more recently) PCR for definitive diagnosis of *H. pylori* infection (8, 10–19).

Serum ELISA has proven to be a highly specific and sensitive noninvasive assay to diagnose *H. pylori* infections in many human studies. The significant increases in levels of IgG *H. pylori* antibody in two experimentally infected cats were encouraging, but the low antibody titers in the remaining two infected cats suggest that the IgG serum antibody response to *H. pylori*, as determined by our ELISA, may not provide a reliable test for *H. pylori* infection in cats. Determination of *H. pylori* IgG antibody levels in sera obtained on a longitudinal basis from naturally infected cats as well as the use of other defined *H. pylori* antigens in ELISAs should help clarify this issue.

Our experimental studies have important implications for future surveys to ascertain *H. pylori* status in animals. A systematic and detailed analysis involving several diagnostic criteria may be required to ascertain the true *H. pylori* status of pet cats. Also, our finding of *H. pylori* in the cardia of all four cats suggests that this site, because of its histological similarities to the antrum, provides an additional nidus for *H. pylori* colonization in the cat. This parallels results of a recent study with humans in which *H. pylori* was as prevalent in the cardia as in the rest of the stomach for one group of patients. In addition, the intensity of inflammation in the cardia mimicked that seen in the antrum (17). To augment the less sensitive routine diagnostic tests, a highly specific and sensitive PCR assay for *H. pylori* in feline gastric tissue may be required to diagnose *H. pylori* (8, 25). Saliva and feces may also be screened for *H. pylori*. We have detected *H. pylori* by PCR in feces of cats naturally infected with *H. pylori* (44a). Similar findings have been noted recently for human feces, in which *H. pylori* has been identified by both culture and PCR (29, 48). Care also must be exercised when screening histological samples for GHLOs. The large gastric spiral organisms elicit a histological gastritis in cats that is similar to that induced by *H. pylori*. Large gastric spiral bacteria are easily demonstrated histologically (22, 32). As is the case with nonhuman primates and humans, however, in which *H. pylori* can occasionally cocolonize with the large gastric spiral bacteria, *H. pylori* in cats may be more difficult to visualize by special tissue stains than GHLOs and may be missed with casual review (6, 22, 46).

Neither of the two control cats had gastric biopsy or necropsy samples which were positive for *H. pylori* during the course of the experiment. Thus, the experimental production of gastritis in naive cats, along with the demonstration of a persistent infection caused by the *H. pylori* strain used in the inoculum, fulfills Koch's postulates. In an attempt to mimic housing conditions used for the cats naturally infected with *H. pylori*, we grouped the four experimentally infected cats in one enclosure. This may have enhanced the reexposure of the cats to *H. pylori* by the other cats in the room. Interestingly, the

lymphofollicular gastritis and subglandular and superficial diffuse lymphoplasmacytic gastritis, located primarily in the antrum, were indistinguishable from the gastritis noted for cats naturally infected with *H. pylori* (20, 21). Lesions in one experimentally infected cat also had a diffuse infiltration of polymorphonuclear cells and a moderate number of eosinophils in the subglandular area of the antrum. This histological feature, i.e., active chronic gastritis, is a typical feature of disease caused by *H. pylori* in humans (18, 33, 34, 37). Also, for one cat, as has been noted occasionally for humans naturally infected with *H. pylori* (33, 34), moderate numbers of eosinophils were noted in the gastric lamina propria and subglandular areas. The antral distribution of the lesions was also consistent with that seen with *H. pylori* gastritis in humans. The multifocal lymphoplasmacytic follicles noted in these cats are common in gnotobiotic pigs experimentally infected with *H. pylori* and in gnotobiotic dogs infected with either *Helicobacter felis* or *H. pylori* (30, 35, 44). Ferrets infected with *Helicobacter mustelae* and dogs, cats, and rodents infected naturally or experimentally with GHLOs also have this type of gastric lesion histologically (11, 12, 14, 34, 35, 41). Furthermore, lymphoid follicles are a common feature of *H. pylori*-associated gastritis in humans (16, 17, 45). In one recent study, lymphoid aggregates in a biopsy specimen were virtually always associated with *H. pylori*-associated active chronic gastritis (15). In another survey, the lymphoid follicles in *H. pylori*-infected individuals were much more common in the antrum than in the cardia or body (16), paralleling our findings for cats.

Several case reports of GHLO infections in humans have suggested pet animals as possible sources of infection (22, 31, 32, 38, 49, 51). Recently, an epidemiological study supported the hypothesis that domestic pets were a source of zoonotic spread of GHLOs; however, the data focused on *H. felis* and *Helicobacter heilmannii*, not on *H. pylori* (46). This experiment and our earlier report identifying cats naturally infected with *H. pylori* indicate that *H. pylori* colonizes the cat stomach, produces persistent infection, and elicits a profound gastritis and, in selected cats, a systemic *H. pylori* IgG antibody response. Studies are now ongoing to establish whether *H. pylori* strains isolated from humans can produce results similar to those described above. Furthermore, the presence of *H. pylori* in cat feces increases the likelihood of zoonotic transmission because of human exposure to cat feces during the routine cleaning of litter boxes. Certainly other fecally transmitted bacteria, e.g., *Salmonella* spp. and *Campylobacter jejuni*, as well as the protozoan *Toxoplasma gondii* are known to infect humans by zoonotic transmission (5, 10). Because of these findings, surveys to ascertain the prevalence of *H. pylori* in pet cats are under way to establish the importance of the cat as a reservoir host for the zoonotic transmission of *H. pylori.*

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